Proposal to implement Action 182 of the NOAA Fisheries 2000 BiOP

Relative reproductive success of hatchery-origin and wild-origin steelhead spawning naturally in the Hood River

Applicant and principle investigator:
Dr. Michael Blouin, Associate Professor
Dept. Zoology, Oregon State University
Corvallis, OR 97331
Tel: 541-737-2362
Fax: 541-737-0501
blouinm@science.oregonstate.edu

Date: 4/10/03

TABLE OF CONTENTS

PROGRAM SUMMARY

The Hood River supports two populations of steelhead, a summer run and a winter run. They spawn only above the Powerdale Dam, which is a complete barrier to all salmonids. Since 1991 every adult passed above the dam has been measured, cataloged and sampled for scales. Therefore, we have a DNA sample from every adult steelhead that went over the dam to potentially spawn in the Hood River from 1991 to the present. Similar numbers of hatchery and wild fish have been passed above the dam during the last decade. During the 1990's "old" domesticated hatchery stocks of each run (multiple generations in the hatchery, out-of-basin origin; hereafter H_{old}) were phased out, and conservation hatchery programs were started for the purpose of supplementing the two wild populations (hereafter "new" hatchery stocks, H_{new}). These samples give us the unprecedented ability to estimate, via microsatellite-based pedigree analysis, the relative total reproductive success (adult to adult production) of hatchery and wild (W) fish for two populations, over multiple brood years, and for multiple generations through F2's. Furthermore, we can compare the relative success of two "old" hatchery stocks vs. wild fish, and two "new" hatchery stocks vs. wild. Our preliminary analyses of samples from the 1990's show that individual parents of "old" hatchery stocks have much lower total fitness than wild fish, but that "new" stocks have fitness that is slightly lower than that of wild fish. We also found that the relative fitness of the three types of parental crosses was H_{new} x H_{new} < H_{new} x W =< W x W. All three types of crosses produce enough returning adults that we can use their offspring (F2's) to estimate the fitness of F1's as a function of the fraction of their genome that has been through a hatchery. Here we propose continuing sampling and genotyping through the rest of this decade in order to generate an almost 20 year pedigree for the two runs. From this pedigree we will obtain estimates of the mean and year-to-year variance in the relative reproductive success of hatchery vs. wild fish, parameter estimates that are critical for predicting the effects of hatchery supplementation on wild steelhead populations. We will also use the pedigree to ask a number of other applied and basic questions. For example, we will estimate the heritabilities and genetic correlations among various phenotypic traits in hatchery and in wild fish, and we will examine the effects of supplementation on the effective size of the population over time. These data will be very relevant to the question of whether successful reproduction by hatchery fish in the wild might be having negative genetic effects on the wild population.

PROGRAM DESCRIPTION
Overall goals
Estimate the reproductive success (total fitness defined as adult-to-adult production) of hatchery-origin steelhead relative to that of wild-origin steelhead that have been spawning in the Hood River. Estimate this difference using "old" hatchery stock vs. wild, and "new" hatchery stock vs. wild. Do the comparison for multiple brood years in order to estimate the year-to-year variance in the parameters.

Background on the basin and stocks
The Hood River supports wild runs of winter and summer steelhead. Breeding areas for winter and summer fish are segregated, with summer fish breeding in the West Fork of the Hood River and winter fish breeding in the remaining tributaries (Fig. 1). The

Powerdale Dam at mile 4.0 on the river is a complete barrier to migrating salmon. Facilities include an adult trap and sorter built by BPA. The trap is used for all broodstock collection, for monitoring hatchery and wild adults, and for controlling entry of hatchery fish into natural production areas (a photo of the dam and of the inside of the fish handling facility can be seen at http://oregonstate.edu/~blouinm/Hood%20RiverProject_files/slide0001.htm). This facility provides the unique opportunity to handle the entire population of returning adults every year. Since 1991 every adult passed over the dam has been catalogued, measured and sampled for scales. Traps for sampling juveniles have been in place in the main stem and at the outlets of all the main tributaries since the mid 1990s. The dam is scheduled to be removed in 2010, although the actual date has not yet been determined. The dam is being kept in place until that time in order to facilitate several ongoing research projects in the basin, including this one. We plan to base this study on samples collected through at least the 2008-2009 run year.

Winter run hatchery stock 13 (a domesticated, out-of-basin, multi-generation hatchery stock from Big Creek) was previously stocked in the basin but was phased out in 1991. It was replaced by conservation hatchery stock 50, which uses wild Hood River broodstock each generation and was implemented for the purposes of supplementing the wild winter population. The first generation of stock 50 adults began returning in appreciable numbers in 1995 (Fig. 2). Since then the number of H_{new} fish passed above the dam has been limited to no more than the number of wild fish passed (Table 1). This protocol created an ideal opportunity to evaluate the relative reproductive success of each type of fish spawning in the wild.

Summer run hatchery stock 24 (a domesticated, out-of-basin, multigeneration hatchery stock from Skamania) was phased out in 1998 and replaced by summer conservation hatchery stock 50 in 1998. The protocols for this summer-run supplementation program are the same as for the winter run program. Skamania stock 24 are still planted below the dam to provide a sports fishery, but none are allowed above the dam. Here we use the abbreviation H_{old} to refer to "old" hatchery stocks 24 and 13, and H_{new} to refer to the "new" conservation hatchery winter stock 50 and summer stock 50.

Project Coordination
The genetics pedigree work will be carried out by Michael Blouin at Oregon State University. This project is coordinated with the Hood River steelhead hatchery and research project, funded by Bonneville Power Administration and administered and implemented by the Oregon Department of Fish and Wildlife (Rod French and Erik Olsen, supervisor and database manager).

Figure 1. Study site. Powerdale dam is a complete barrier to salmonids at mile 4.0.
Summer steelhead breed in the West Fork, while winter steelhead breed in the Middle
Fork, East Fork, and Neal Creek. Juvenile traps are located just above the dam and at the
base of each of the main branches within the system.

Table 1. Summary of numbers of wild (unclipped) and hatchery (clipped) fish passed above
Powerdale Dam. These counts do not include fish taken for broodstock, which we also
genotyped. Run year refers to the fall season (e.g. the 91-92 run year fish began arriving fall of
1991and continued arriving into 1992). In 1992 the H_{old} stock 13 program was phased out for
winter run, and the winter run conservation hatchery program was begun. Those H_{new} winter
stock 50 fish began returning to spawn in the wild in 94-95. The old summer stock 24 program
was phased out and the new summer run conservation hatchery program was begun in 1998.
Those H_{new} summer fish began returning in 01-02. Highlighted cells have been genotyped.
The rest will be typed as part of the work proposed here. The key parental years for which we
have preliminary comparisons of hatchery and wild parental fitness are highlighted in bold.
For example, to compare the fitness of the 212 W and 161 H_{new} winter fish that went upstream
in the 95-96 run year, we matched them against all *unclipped* winter fish that returned in 1998
to 2001 and whose scale ages indicated they were born in 1996 (i.e. from the 95-96 run year
parents; 7see also Fig. 2) (We did not genotype the H fish from 98-01 for our preliminary
analyses because, of course, they can't be the offspring of fish that bred in wild).

WINTER RUN

run year	wild fish passed	hatchery fish passed: H_{old} stock 13	hatchery fish passed: H_{new} stock 50
91-92	**632**	**273**	
92-	350	5	
93-	304	2	
94-	160	0	6
95-	**212**	**0**	**161**
96-	**242**	**0**	**249**
97-	184	0	162
98-	258	0	186
99-	875	0	222
00-	883	0	657
01-	954	0	682

SUMMER RUN

run year	wild fish passed	hatchery fish passed: H_{old} stock 24	hatchery fish passed: H_{new} stock 50
92-93	489	1722	
93-	243	1105	
94-	218	1635	
95-	**132**	**520**	
96-	**182**	**1312**	
97-	65	447	
98-	100	4	
99-	148	0	
00-	179	0	
01-	415	0	127
02- (03 to date)	540	0	492

Figure 2. Example of when offspring of each winter run parental breeding year are expected to return. Circles represent run years (e.g. 92-93 means the fish that returned in fall of 1992 through winter of 1993, and spawned in 1993. For simplicity, we will say spawning took place and their offspring were born in 92). Lines and numbers represent the percentage of babies born in a given breeding year that will return in each of the subsequent years. For example, 6% of the offspring born in the wild in 96 are expected to return to Powerdale Dam as unmarked adults in year 98, 61% are expected to return in year 99, and so on (the timing of return of hatchery fish is different from that of wild fish). Solid lines represent hatchery fish, dotted lines represent fish born in the wild. These numbers are based on age distributions of wild adults returning to Powerdale dam. Descendents of the first generation of winter run conservation hatchery stock 50 fish are illustrated as an example. Those hatchery fish spawned in nature mostly in years 95 and 96. Their F1 offspring are almost all returned by 01, and > 90% of F2's born in 99 and 00 are back by 05. The study proposed here involves genotyping samples collected through the 08-09 run year in order to insure a large sample of F2's from several F1 breeding years.

Specific questions to be asked

Adult offspring returning to the dam will be matched back their parents that were sampled in previous years (Fig. 2, Table 1). From these data we will answer the following questions.

(1) What is the mean and year-to-year variance in relative reproductive success (adult to adult production) of hatchery-origin (H_{new}) and wild-origin (W) fish that spawn naturally in the Hood River each year?

> The relative rate of adult-to-adult production by H_{new} and W fish is the key unknown parameter needed for predicting the demographic effects of hatchery supplementation on wild populations. Year-to-year variation in numbers of spawners and in environmental conditions might cause that parameter to vary from year to year. For example, it is well known that the fitness differences between inbred and outbred organisms are exacerbated as the environment becomes more stressful (Jimenez et al., 1994; Cronkrak and Roff, 1999). Here we will measure the parameter (H_{new}:W fitness) for each of ten brood years (run years 95 to 04) of winter fish and for three brood years (02 to 04) of summer fish (see Fig. 2 and Table 2). In addition to estimating a year-to-year variance, we will also ask if any environmental conditions or hatchery program procedures correlate with years of high or low hatchery relative fitness (e.g. crowding levels on the spawning grounds, relative numbers of H and W breeders, previous ocean conditions, when or where hatchery fish were released, and so on). These data may give valuable insight into how we might improve hatchery programs to maximize successful supplementation.

(2) Do F1 progeny (born in the wild) of H_{new} x W, H_{new} x H_{new} and W x W winter run parents differ in their production of F2 progeny?

> We know from our preliminary analyses that all three types of matings occur on the spawning ground, and that all three types of mating produce offspring that return to spawn as adults. F2 offspring of those winter F1s that spawned in the late 1990s are now returning (see Fig. 2). If we continue sampling through the end of the decade we will be into the F3 generation for some winter fish, and should have a large number of returned F2s from multiple brood years with which to test the relative fitness of different types of F1s (Fig. 2).

(3) Are "new" hatchery stocks closer in fitness to wild fish than "old" hatchery stocks?

> Theory and substantial circumstantial evidence suggest that "old" hatchery stocks will have substantially lower total fitness than "new" hatchery stocks in the wild (Lynch and O'Hely, 2001; Fleming and Petersson, 2001). However, there has never been a direct test of this hypothesis, nor are there any empirical data on how much better the "new" stocks should perform. Here we have the unique opportunity to test the relative fitness of H_{new} vs. W and H_{old} vs. W in the same two populations in the same river. For winter run we have one run year of H_{old} vs. W (91), and for summer run we will have six years (92-97) (Table 1). Our preliminary data suggest that H_{old} are indeed worse than H_{new}.

Parameters vs. parameter estimates
For each run year we are interested in making inference about the fitness of the anadromous hatchery and wild fish that went above the dam. Because we sample all the fish at the dam each year, the fitness values we obtain for each type of parent (H or W) in a given run year are the parameters for that year, not estimates of the parameter. In other words, the parents that return each run year *are* the population of inference, not a sample from some larger population of hatchery and wild fish to which we wish to make inference for that year (the fitness of an individual fish may be measured with error owing to mis-assignment of offspring, but that is an issue of precision of measurement). On the other hand, the fitness estimates obtained for a given year can be considered to be a sample from some larger universe of run years. Because the ultimate goal here is to estimate H:W relative fitness for use in modeling conservation hatchery programs in general, the key values of interest are the mean and variance of H:W fitness among run years. So our main focus here is in measuring H:W fitness in as many run years as possible.

Finally, note our focus on the production of adults rather than juveniles as the measure of fitness. Although we have scales from large samples of smolts leaving the basin each year since 1994, we are not proposing at this time to genotype them and match them to parents (although this could always be done in the future). The reason is we have concerns over whether a sample of smolts is truly random with respect to the families that produced them (owing to family effects on when they outmigrate, and on where in the system they were caught). In contrast, adults that returned to the dam are, like their parents, the population of interest and not sample from it. Again, the production of adults is the true measure of the demographic impact of a hatchery supplementation program on a wild population, and so is ultimately what we want to measure.

Methods
(1) Sampling:
All handling of fish, phenotypic data collection, and sampling of scales and fin snips is done at the Powerdale Dam by Oregon Department of Fish and Wildlife staff. All fish approaching the dam are shunted into a trap and lifted into a building built specifically for the purpose of handling these fish. After being measured and sampled, each fish is either recycled downstream (e.g. extra hatchery fish), taken as broodstock or put above the dam to continue on to the spawning grounds. Sampling and database management protocols have been in place since project inception. Thus, we have an extensive database on the size, run timing, age and freshwater residency (from scales), gender, fin clip and disposition (i.e. taken for broodstock, recycled, etc…) of every fish for which we also have pedigree data.

(2) Molecular Methods:
We use a standard chelex protocol to extract DNA from fin snips or scales. Note that we obtain high quality DNA template from the scale samples, even those from the early 1990's. All extractions are done in 96-well plates.

From an initial set of 27 microsatellite loci that are known to work well in steelhead, we chose a set of eight loci based on the criteria that they amplify well, can be scored unambiguously in two sets of four multiplexed loci, and lack high-frequency null alleles. These eight loci provide a total power to reject a false parent-offspring pair via simple exclusion of 0.99993 (Table 2). Furthermore, our ground truthing experiments (see below) demonstrate a very low empirical rate of false parentage inclusion, and high power to exclude all but the true parents.

Template plates are pooled into 384-well plates for PCR, and those are pooled into four-locus, 384-well plates for multiplex scoring on an ABI 3100 16-channel capillary electrophoresis system. We use a Hydra-96 liquid handling robot (Robins Scientific) for all pipetting procedures involving plates (i.e. for all procedures following the initial handling of the scale or fin snip). Adding this device to our lab has cut sample handling errors down to virtually zero.

Using the above procedures we have now successfully genotyped over 9,000 steelhead at those eight loci. All procedures are optimized and automated in my lab, so we should have no problem continuing the work.

Table 2. Summary statistics for the eight loci we use (from a sample of winter run; summer fish have similar levels of diversity).

Locus	Number of Alleles	Expected Heterozygosity	Exclusionary Power
Omy1001	22	0.91	0.666
Omy1011	13	0.90	0.641
Omy77	16	0.88	0.599
One108	21	0.93	0.729
One2	34	0.95	0.800
Rt191	21	0.93	0.723
Ssa407	20	0.90	0.660
Str2	32	0.96	0.814

(3) Data analysis:
The eight-locus genotypes are merged with ODFW's database in Microsoft Access. For each returning fish (putative offspring) we search for it's mother and father in the year in which it was born (based on scale aging), plus or minus one year to allow for aging errors. From preliminary matching tests using a wider window we found only a few percent of fish are mis-aged by one year, and none are mis-aged by two years. For parent-offspring matching we use standard likelihood-based parentage analysis with an empirically-determined genotyping error rate (Marshall et al., 1998). Note that the presence of trout or precocious parr in the system is not be a problem, even if they obtain some matings with anadromous parents. Our inference is to the average fitness of anadromous H and W parents, where fitness is defined as production of returning anadromous adults. Only offspring that assign to parents are relevant to the study, and we have large sample sizes of those.

(4) Ground truthing:
All fish taken for broodstock are also genotyped. Therefore, as a form of ground truthing we ran fin-clipped returning adults from four brood years through the parentage analyses. For these analyses the fish taken for broodstock were included in the pool of wild potential parents. Ninety-six percent of the clipped returning fish were unambiguously matched back to a single mother-father pair in their expected brood year, and in every case our hatchery records show that that male-female pair was indeed crossed in the hatchery. The remaining unassigned, clipped offspring mismatch all potential parents at multiple loci and so are probably stray hatchery fish from out of the basin. Clipped and unclipped fish were treated identically during all stages of data collection. Therefore, we should have the same power to find the parents of unclipped and unclipped returnees if their parents are in the parent pool.

Preliminary Results
This project was started two years ago with two small subcontracts from ODFW and a supplemental contract from BPA. That funding, which has now ended, was to set up the project and analyze H vs. W fitness for winter fish spawning in 91, 95 and 96, and for summer fish spawning in 95 and 96 (bold highlighted rows in Table 1). We just finished the genotyping and have conducted a preliminary analysis of the results, which I summarize below and in Tables 3 and 4.

(1). Analyses for individual fish of each sex:

Winter run: H_{old} vs. W and H_{new} vs. W
The 91 year gives a comparison of H_{old} vs. W, and the 95 and 96 years give comparisons of H_{new} vs. W. In 91 the H_{old} had 35% the fitness of wild fish. In 95 the H_{new} had 85% the fitness of wild fish, and in 96 the H_{new} had 85-90% the fitness of wild fish (Table 3). These results are consistent with the opinion that "new" hatchery stocks perform much better than "old" hatchery stocks. They also show, however, that the H_{new} fish are not equal to wild fish.

Summer run H_{old} vs. W
We matched summer-run offspring back to their putative parents that spawned in the 95 and 96 run years. Both years involve comparisons of H_{old} vs. W. The relative fitness of H_{old} vs. W was 45-54% in 95 and only 17-30% in 96 (Table 3). In this case it is interesting that the relative performance of the H_{old} fish was lowest in 96 when almost twice as many summers were on the spawning grounds. These results also show how variable the relative fitness of hatchery fish may be from year to year.

Table 3. *Preliminary* analysis of average number of offspring produced per potential spawner of each type in each run year. n = number of adults collected at the dam (potential spawners) and genotyped. "Avg. # offspring per adult" = average fitness (number of offspring matched back) per potential spawner. "H/W relative fitness" is the average # of offspring per hatchery adult divided by the avg. # per wild adult. Discrepancies between the total number of fish in this table and in Table 1 in some years result because (1) for this analysis we excluded hatchery fish that had been recycled once or twice before being put above the dam (because that may have affected their fitness), and (2) we excluded a few parents for whom we did not have complete genotypes.

WINTER RUN

Run Year	H_{old} (stock 13)			Wild			
1991-92	n	Avg. # offspring per adult		n	Avg. # offspring per adult		H/W relative fitness
males	165	0.24		247	0.68		0.35
females	99	0.23		379	0.68		0.34

Run Year	H_{new} (stock 50)			Wild			
1995-96							
males	90	3.66		78	4.31		0.85
females	65	4.42		132	5.05		0.85

Run Year	H_{new} (stock 50)			Wild			
1996-97							
males	95	2.28		93	2.54		0.90
females	153	2.08		148	2.47		0.85

SUMMER RUN

Run Year	Hold (stock 24)			Wild			
1995-96							
males	211	0.72		44	1.34		0.54
females	297	0.45		86	1.01		0.45
Run Year	Hold (stock 24)			Wild			
1996-97							
males	474	0.45		61	1.48		0.30
females	766	0.20		121	1.18		0.17

(2) Analyses of parental pairs (performance of H_{new} x H_{new}, H_{new} xW and WxW crosses):
We could estimate the proportions of each type of observed cross expected if H and W fish mate randomly, and then compare those proportions to the observed proportions of parental pairs we detected of each type. But because we can't count pairs that left no surviving offspring, there is no way to disentangle non-random mating from differences in parental fecundity or offspring survival (you would need to observe matings to do that). If we restrict our analysis to pairs that left *at least one surviving offspring*, we can calculate the relative fitness of each type of cross for that truncated dataset. Any difference here is necessarily owing to offspring survival or parental fecundity because we have restricted the inference to those fish that, by definition, mated. This analysis almost certainly underestimates the fitness differences among the three types of pairs because we have no zero-offspring class. Nevertheless, even for this restricted dataset our preliminary results show that HxH crosses did worse that HxW or WxW crosses (Table 4). Of more importance, however, our preliminary results show that (1) all three types of crosses occur in the wild, and (2) they produce surviving F1 offspring in large enough numbers that we will be able to estimate the relative fitness of those three types of F1 (via matching them with F2's that return in later years).

Table 4. *Preliminary* analysis of average number of offspring per type of parental pair (HxH, HxW or WxW) for pairs *that left at least one offspring*. n = number of that type of pair unambiguously identified as leaving at least one offspring.

WINTER RUN

Type of cross	1991 Avg. # (n)	Fitness of the cross relative to W x W	Type of cross		1995 Avg. # (n)	Fitness of the cross relative to W x W	1996 Avg. # (n)	Fitness of the cross relative to W x W
W x W	1.33 (87)	1.00	W x W		2.53 (72)	1.00	1.54 (57)	1.00
W x H_{old}	1.09 (11)	0.82	W x H_{new}		2.05 (78)	0.81	1.58 (90)	1.03
H_{old} x H_{old}	NA (0)	NA	H_{new} x H_{new}		1.29 (21)	0.51	1.42 (38)	0.92

SUMMER RUN

			Type of cross		1995 Avg. # (n)	Fitness of the cross relative to W x W	1996 Avg. # (n)	Fitness of the cross relative to W x W
			W x W		1.75 (4)	1.00	1.46 (13)	1.00
			W x H_{old}		1.29 (17)	0.74	1.24 (50)	0.85
			H_{old} x H_{old}		1.0 (10)	0.57	1.05 (63)	0.72

Other questions of interest

In this proposal I have responded only to the specific request of BiOP Action #182 for projects to estimate the relative reproductive success of hatchery fish vs. wild. However, with a two-decade pedigree on two populations (summer and winter) we can also ask many other interesting applied and basic questions. These topics include, but are not limited to:

(1) Selection to maintain the difference between summer and winter runs:
What is the rate of hybridization between the runs? What are the phenotypes (run time, size, freshwater residency) and actual fitnesses of any hybrids?

(2) Selection on measurable phenotypic traits:
We can use standard selection gradient analysis (Lande and Arnold, 1983) to analyze fitness as a function of body size, run time, age and freshwater residency (known from scales), after controlling for hatchery/wild genetic background.

(3) Quantitative genetic parameter estimation:
From our pedigrees we can estimate the heritabilities of, and genetic correlations among any measurable phenotypic traits. We can also estimate the average breeding value for each trait in individuals of HxH and WxW genetic background, in order to test whether genetic changes in the hatchery, and subsequent mating with wild fish, could be changing phenotypic distributions in the wild population (Ford, 2001).

(4) Parental contributions of resident, non-anadromous fish
We sample all potential breeding adults passed over the dam, and we know from our ground truthing experiments the expected rate of mismatching owing to experimental error. Therefore, unassigned offspring are either wild strays from out of the basin, or were parented by resident fish (non-anadromous *O. mykiss*, or precocious parr). First, we will estimate the rate of such resident contributions. Second, we will use likelihood methods (Rannala and Mountain, 1997) to attempt to determine the most likely source of missing parents of offspring that only match to a single known parent, and whether fish lacking both parents could be immigrants from adjacent steelhead populations. Again, because we sample all anadromous parents, the Hood River is an ideal system in which to ask questions about the rate of parentage from resident fish and about the sources of those fish.

(5) Effective size estimation
From the pedigrees we can obtain direct estimates of the effective size (*Ne*) of each population over time. These data will be used to estimate the impact of hatchery programs on the effective size of the wild population and to provide basic parameter estimates such as the variance in family sizes (number of returning adults) for hatchery broodstock, for H fish in the wild, and for W fish in the wild. These are important parameters that are unknown for most populations and can be very useful for estimating *Ne* and the effects of supplementation in other steelhead populations (e.g. *sensu* Ryman et al., 1995). We can also use our system to evaluate the accuracy of indirect methods for

estimating effective size (e.g. Waples, 2002; Anderson et al., 2000). If the indirect methods give very different values from the pedigree-based estimates, then we can ask what assumptions of the indirect methods cause the difference. Note that because of our ability to sample all potential anadromous parents, we can take into account the contributions of non-anadromous, resident fish in our calculations.

Uniqueness of this project
Although there are a number of projects underway in the Northwest that are designed to examine H:W relative fitness, ours is unique in several aspects.

(1) We genotype all anadromous adults each year, not just a sample of them. Thus, for example, we can measure the H:W fitness parameter directly, and we can study the sources of unmatched offspring without the complication of missing anadromous parents.

(2) We are using adult-to-adult production as our measure of fitness. This is the true measure of whether hatchery supplementation programs work. Estimates of the mean and variance in this parameter are what is needed to model the effects of hatchery supplementation in other systems, and that is what our project will provide.

(3) The project started twelve years ago, so we now have useful data on H_{new} vs. W and H_{old} vs. W for several breeding years. With every passing year we add another run year to the dataset. Soon we will have enough observations to provide a good estimate of the year-to-year variance in the key parameters. Because steelhead have a relatively long lifecycle (Fig. 2), no other project that started recently will be able to produce the same information for steelhead until well past 2010. By then we will have a large dataset on parental fitnesses (H or W origin) and F1 fitnesses (wild born of HxH, HxW or WxW origin).

(4) Our data will be applicable to steelhead populations in general, not just to the Hood River or to steelhead in that region of the Columbia basin. First, we are dealing with a relatively healthy population of each run (usually dozens to a few hundred wild fish each year), not some tiny population. This feature also gives us a large sample size each year for the H:W comparison. Second, we are studying two independent runs, winter and summer. Third, we will be able to provide estimates of year-to-year variation in the key parameters, not just provide one or two point estimates.

(5) We will examine the relative fitness of "old" and "new" hatchery stocks vs. wild in the same two runs in the same river. This situation provides a more compelling test of the hypothesis that "new" stocks perform better than "old" stocks than, say, a meta-analysis of different projects on a variety of species in different drainages. Indeed, our initial data strongly suggest that, as most people suspect, "old" stocks have poor fitness while "new" stocks do much better relative to wild (Table 3). A few more run years of data should settle the issue.

References Cited

Anderson, E.C et al. 2000. Monte Carlo evaluation of the likelihood for N_e from temporally-spaced samples. *Genetics* 156: 2109-2118.

Cronkrak, P. and D. Roff. 1999. Inbreeding depression in the wild. *Heredity* 83:260-270.

Fleming, I.A. and E. Petersson. 2001. The ability of released hatchery salmonids to breed and contribute to the natural productivity of wild populations. *Nordic Journal of Freshwater Research* 75:71-98.

Ford, M.J. 2001. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* 16:815-825.

Jimenez et al. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* 266:271-273.

Lande, R. and S.J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 36:1210-1226.

Lynch, M and M. O'Hely. 2001. Supplementation and the genetic fitness of natural populations. *Conservation Genetics* 2:363-378

Marshall et al. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639-655.

Rannala, B. and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci*. USA 94: 9197-9221

Ryman, N.et al. 1995. Supportive breeding and variance effective population size. *Conservation Biology*. 9: 1619-1628.

Waples, R. 2002. Effective size of fluctuating salmon populations. *Genetics* 161:783-791.

Work Plan and Timeline

Yr 1, June 2003- May 2004: Hire postdoc and technician, initial training of personnel. This first year we will focus on formal analysis of the first two year's pedigree data, and begin genotyping the backlog of samples that have not been run yet from the 1990's (unshaded rows in Table 1). Publication of initial results from the first two years' data.

Yr 2, 2004-2005: Continue genotyping. Produce report on data from run years genotyped to date. Final data on H_{old} vs. W parental fitnesses in summer run now available. Analysis of effective size data, publications.

Yr 3, 2005-2006. Continue genotyping. Quantitative genetics analysis and publications.

Yr's 4, 5, 6, 7: 2006-07 to 2009-10: Continue genotyping through the last year of samples planned for this project (08-09). Analysis of F1 relative fitnesses.

Yr 8 2010-2011. Final analyses, reports and publications.

Budget (approximate estimates rounded to nearest $1,000)

Yr 1 $215,000 (Personnel: Postdoc, technician, PI summer salary, plus OPE = $122,000; Supplies $30,000, University overhead at 41.5% = $63,000).

Yr2 $266,000 (same personnel plus graduate student, supplies for a full year of genotyping).

Yrs 3 to 7: $277,000 to $323,000 (same as yr 2 plus 4% inflation per year)

Yr 8: $135,000 (Final year of analysis, writing reports and manuscripts; inflation adjusted Postdoc salary, PI summer salary; OPE and overhead).

TOTAL PROJECT: $2,125,000.

QUALIFICATIONS OF PARTICIPANTS

(1) Summary of Personnel and duties
        1. Michael Blouin – Principal investigator, lab manager. Supervises entire project; data analysis, publications
        2. Postdoc – lab work, data analysis, publications
        3. Technician – lab work, database management
        4. Graduate student – Assists technician and postdoc; lab work, database management.

(2) Postdoc and technician:
Because the funding I had for the initial two-year project has ended, the technician and postdoc I originally hired have now taken other positions. If the work I propose here is funded, I will hire a new postdoc and technician this summer. The main qualifications for the postdoc will be a good background in population and quantitative genetics, plus basic molecular skills. I found in the first two years of this project that the sample management and error checking of such large databases is more involved than the actual genotyping, which is routine. Therefore I will look for a technician who has experience with data management as well as the basic molecular biology skills necessary for microsatellite analysis.

(3) Principal Investigator

My original training was in quantitative genetics, but my current research centers around the use of DNA methods to ask questions about the causes and consequences of genetic structuring in natural populations. I am particularly interested in the application of methods for reconstructing pedigrees in natural populations. I have been teaching Genetics, Population Genetics and classes in Molecular Methods for over ten years at OSU and at two other Universities. My lab is currently set up for high-throughput microsatellite genotyping. In less than two years we (myself, a post doc, a technician, and a part time graduate student) developed the molecular protocols for this project and genotyped over 9,000 fish. All the protocols are now standardized, so it should be no problem to train new personnel and pick up where we left off.

(4) Collaborators

This work is being done in collaboration with the Oregon Dept. Fisheries and Wildlife, who run the Powerdale facility, provide the tissue samples each year, and maintain the main database of fish ID's and associated data. Contacts: Erik Olsen (pelton@gorge.net) and Rod French (rfrench@odf.state.or.us) (ODFW, 3561 Klindt Dr., The Dalles, OR 97058, 541-296-8045).

(5)Agency Contacts for previous work on this project

My agency contacts for the first two years of funding for this project were Kathryn Kostow (Kathryn.E.Kostow@state.or.us; ODFW, 17330 SE Evelyn St., Clackamas, OR 97015; 503-657-2000 ext. 247) and Tom Morse (temorse@bpa.gov; BPA, 905 NE 11th Ave., Portland, OR 97208-3621, 503-230-3694).

MICHAEL S. BLOUIN – brief CV

Dept. Zoology, Oregon State University
Cordley 3029, Corvallis, OR 97331-2914
Tel: (541) 737-2362; Fax (541) 737-0501;blouinm@science.oregonstate.edu

http://www.onid.orst.edu/~blouinm/index.htm

A. EDUCATION AND EMPLOYMENT

Education
12/89	PhD/biology Florida State University, Advisor: Joseph Travis
12/86	MS/biology Florida State University, Advisor: Daniel Simberloff
5/82	BA/Interdisc. University of Virginia, Echols Scholar, BA w/high distinction

Professional positions
  6/01-pres.   Associate professor, Dept. Zoology, Oregon State University
  7/95- 5/01.   Assistant professor, Dept. Zoology, Oregon State University
  1/94 - 5/95   Assistant professor, Dept. Biology, University of South Florida
  7/93 -12/93   Visiting researcher, UC Davis Bodega Marine Lab, CA
  1/92 - 6/93   Assistant Professor, Dept. Biology, Sonoma State University, CA
  6/90-12/91   Post-doc, Dept. Infectious Diseases, University of Florida
  1/90-5/90   Data Analyst, U.S. Fish and Wildlife Service, Gainesville, FL

B. TEACHING
Current teaching schedule
Fall quarter: Bi311, General Genetics (4 cr)
Winter: Gen430/530 (Population Genetics) (3 cr), Zoo582 (Molecular Methods in Ecology and Evolution) (3 cr)
Spring: graduate seminar in Evolution (1 cr)

C. EXAMPLE PUBLICATIONS
Blouin, M.S. Advances in pedigree reconstruction in natural population. *Trends in Ecology and Evolution*, in review

Hoffman, E. and M.S. Blouin. Evolutionary history of the northern leopard frog: reconstruction of phylogeny, phylogeography and historical changes in population demography from mitochondrial DNA. *Evolution*, accepted pending revisions.

Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Onchorhynchus tschawytscha*) J. *Heredity* 90:281-288.

Blouin, M.S., C.A. Yowell, C.H. Courtney, and J.B. Dame. 1998. Substitution bias, rapid saturation, and the use of mtDNA for nematode systematics. *Molecular Biology and Evolution* 15:1719-1727.

Blouin, M.S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology* 5:393-401.

Blouin, M.S., C.A. Yowell, C.H. Courtney, and J.B. Dame. 1995. Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* 141: 1007-1014.

Blouin, M.S. 1992. Genetic correlations among morphometric traits and rates of growth and differentiation in the green tree frog, *Hyla cinerea Evolution* 46: 735-744.